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SEROLOGICAL OVERLAP BETWEEN LUPUS ERYTHEMATOSUS, RHEUMATOID ARTHRITIS, AND THYROID AUTO-IMMUNE DISEASE

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Circulating auto-antibodies are being reported in an increasing number of diseases, but their role in the pathogenesis of these disorders is far from clear. The clinical interrelationships and the serological overlap between the "auto-immune" diseases is of particular relevance to the understanding of the fundamental disturbances underlying these phenomena. The occasional finding of L.E. cells in patients with thyroid disorders (Wilkinson and Sacker, 1957; Hijmans *et al.*, 1958; Thomas, 1959; White, 1959) and the clinical association of connective-tissue diseases with Hashimoto's thyroiditis (Short *et al.*, 1957; Luxton, 1956; Beare, 1958; Doniach and Roitt, 1959; Heaton, 1959) prompted us to undertake an investigation into the incidence of thyroid antibodies in systemic lupus erythematosus, rheumatoid arthritis, and allied conditions on the one hand, and on the other hand to determine the frequency with which antinuclear factors, as shown by the presence of L.E. cells, fluorescent nuclear staining (A.N.F. test), and nuclear complement fixation occur in patients with diffuse auto-immune thyroiditis.

Since the initiation of this study other authors have reported on the association of Hashimoto's disease with systemic lupus (White *et al.*, 1961), lupoid hepatitis (Mackay and Perry, 1960), Sjögrens's syndrome (Bloch *et al.*, 1960; Bunim, 1961), and rheumatoid arthritis (Buchanan *et al.*, 1961). These recent papers also deal with the presence of circulating thyroid antibodies in patients with connective-tissue diseases (see also Goodman *et al.*, 1960; Jansz, 1960).

Materials and Methods

Clinical Material

The patients were drawn mainly from the three hospitals taking part in this study, but in addition sera were received for antibody tests from many other centres. Three main disease groups were studied, in addition to control groups.

1. *Systemic Lupus Erythematosus (S.L.E.)* (65 cases).—The patients all had systemic manifestations, and we excluded from this group cases of clinical rheumatoid arthritis in which L.E. cell tests were positive. The group consisted of 59 females aged 14–76, with a mean age of 45 years, and 6 males aged 34–76, with a mean age of 55 years. In five of the female patients there was an associated thyroiditis which was clinically overt in three cases, and in the other two was diagnosed only after examination of the thyroid with special function tests. These five cases of combined disease were treated as a subgroup to avoid selection bias.

2. *Rheumatoid Arthritis (R.A.)* (86 cases).—These were all "definite" or "classical" cases as defined by the American Rheumatism Association (Ropes *et al.*, 1959). They included cases with negative latex or sheep-cell agglutination tests, and also cases with positive L.E. cell tests. There were 75 females and 11 males, ranging in age from 24–73, with a mean age of 52 years. Six of the patients had colloid goitre, one was thyrotoxic, and seven cases had primary myxoedema or Hashimoto's disease. The seven cases of combined rheumatoid arthritis and thyroiditis were considered as a subgroup, since they were probably selected from a large rheumatoid arthritis population.

3. *Hashimoto's Disease and Primary Myxoedema* (182 cases).—These were selected for their high antibody levels, but had no overt connective-tissue disease. A few of the patients had thyrotoxicosis with a well-marked superimposed thyroiditis. The female-to-male ratio was 12:1 and the mean age was 51 years.

4. *Controls.*—The incidence of positive reactions for each of the antibody tests employed was previously established in healthy and diseased controls at each of the laboratories, so that different individuals make up the control group for each method.

In addition to these main groups we collected seven cases of combined liver disease and lymphadenoid goitre or myxoedema. These cases were included in the present study in view of the relation of certain cases of hepatitis with lupus erythematosus and the known histological association of thyroiditis with cirrhosis in post-mortem studies (Bastenie, 1937; McConkey and Callaghan, 1960). We also examined 83 cases of Sjögren's syndrome for thyroid antibodies and anti-nuclear factors (in collaboration with J. Heaton and Barrie Jones) and the detailed results of this will be published separately.

Sera.—These were stored at −20° C. for up to three years before the tests were performed. Repeated thawing and freezing of the specimens could not always be avoided.

Serological Methods

L.E. Cell Tests.—Preparations were made according to Zimmer and Hargraves (1952) except for an incubation time of 10 minutes between fragmentation of the blood clot and centrifugation. Where only serum was available the indirect test of Kievits and Schuit (1957) was used.

Nuclear Complement Fixation.—This was performed with chicken erythrocyte nuclei, and the titre was recorded as the reciprocal of the highest serum dilution giving total inhibition of haemolysis using 2 M.H.D. of complement as described previously (Hijmans and Schuit, 1959). Some of the sera were also tested with D.N.A., calf thymus nucleoprotein, and nuclear histone.

Fluorescent Test for Anti-nuclear Factors (A.N.F.).—The indirect (sandwich) technique was used as described by Weir, Holborow, and Johnson (1961). Sections of frozen, unfixed, fresh post-mortem human infant thyroid, or of thyrotoxic thyroid glands obtained at operation, were used as the nuclear substrate. The tests were done at room temperature, but positive Hashimoto and S.L.E. sera were retested at 37° C. (Couchman *et al.*, 1961).

Latex Fixation Test: for Rheumatoid Factor (R.F.).—This was done by the method of Singer and Plotz (1956), using whole serum. Some of the R.A. sera were tested by the Rose-Waaler differential sheep-cell agglutination test (D.A.T.).

Thyroid Antibody Tests.—Three organ-specific auto-antigens are so far known to be implicated in thyroiditis: thyroglobulin, CA2 (second antigen of the thyroid colloid) and the microsomal antigen of thyroid cells. Antibodies to thyroglobulin were detected by the tanned-red-cell (T.R.C.) agglutination test, using formolized sheep cells coated with thyroglobulin (Fulthorpe *et al.*, 1961), and the precipitin test in agar. CA2 antibodies fail to react in these tests and can be detected only by the Coons technique, using frozen thyroid sections fixed in methanol (Balfour *et al.*, 1961). The microsomal antibody was titrated by the complement-fixation test (C.F.T.) using crude thyrotoxic gland extracts and 2 M.H.D. of complement (Roitt and Doniach, 1958). All S.L.E. and R.A. sera reacting with crude thyroid extract were retested with microsomal preparations obtained by differential centrifugation of disrupted thyrotoxic gland homogenates. The organ specificity of the C.F.T. reactions was further checked by cytoplasmic staining of unfixed, frozen thyroid sections with the Coons fluorescent sandwich technique (Holborow *et al.*, 1959). The cytoplasmic fluorescent test is an important check for sera giving high-titre non-organ-specific complement fixation (A.I.C.F. reaction, see below), since microsomal preparations can be contaminated with soluble non-specific antigens which would react in C.F.T. However, A.I.C.F. reactors do not stain the thyroid cytoplasm. In the presence of concomitant bright nuclear fluorescence, it is difficult to judge weak cytoplasmic staining and A.N.F. positive sera should be first absorbed with nuclei, but this has not yet been done.

Auto-immune Complement Fixation Reaction (A.I.C.F.).—This non-organ-specific complement fixation reaction is obtained with human thyroid extracts as well as with human liver or kidney, and the test can be done equally well with rat organs (Gajdusek, 1958; Hackett *et al.*, 1960). All sera giving positive C.F.T. with thyroid were tested in parallel using 1/10 saline extracts of human post-mortem liver and 2 M.H.D. of complement.

Cell Fractionation Studies.—Selected S.L.E. and Hashimoto sera were further tested by C.F.T. with subcellular liver fractions obtained by differential centrifugation (Schneider and Hogeboom, 1950) to determine the heterogeneity of antigens reacting in the A.I.C.F. test.

Results

The incidence of positive serological reactions in the three main groups of patients, and the subgroups of mixed disease together with controls, are shown in Table I. Sera were recorded as having thyroid antibodies when they were positive with one or more of the tests, using specific thyroid antigens.

TABLE I

Clinical Diagnosis	No. of Patients in Series	Incidence of Positive Reactions				
		Thyroid	L.E. Cells	A.N.F.	A.I.C.F. titre ≥ 1 : 16	R.F. Latex Test
Systemic lupus erythematosus	60	15/60 (25%)	59/60 (98%)	59/60 (98%)	22/60 (37%)	20/50 (40%)
S.L.E. + Hashimoto ..	5	5/5	5/5	5/5	4/5	0/5
Rheumatoid arthritis	79	9/79 (11%)	16/79 (20%)	40/79 (50%)	7/72 (10%)	35/64 (55%)
R.A. + Hashimoto	7	7/7	0/7	3/7	1/7	5/7
Hashimoto's disease (uncomplicated)	182	182/182 (100%)	0/110	14/182 (8%)	9/182 (5%)	2/98 (2%)
Controls ..	Composite groups	10%	0%	2%	<1%	2 to 5%

Systemic Lupus

The group of 65 S.L.E. patients (including the five cases with associated thyroiditis) all had positive L.E. cell tests, and the A.N.F. reaction was positive in all but one who was tested when the disease was in remission two years after the L.E. cell test had last been found positive. Thyroid-specific antibodies of high titre were found only in the five cases with diffuse thyroiditis. Of the 60 cases without overt thyroid disorder, 12 gave T.R.C. agglutination titres varying from 1/5 to 1/2500. In all these cases the agglutination reactions could be considered specific since they were inhibited by adding human thyroglobulin to the sera, and were negative when tanned cells coated with sheep thyroglobulin were used.

CA2 antibodies were found in several of the T.R.C. and C.F.T. positive cases and also in three patients having no other thyroid antibodies. In two further patients C.F.T. was positive to a titre of 1/8 with thyroid extract and negative with liver. However, the organ specificity here is not absolutely certain, since the A.I.C.F. reaction is not always obtainable consistently with liver extracts from different individuals. In view of this doubt, these two cases have been excluded, so that definitely thyroid-specific antibodies were found in 15/60 S.L.E. patients without overt thyroiditis. Positive results were entirely confined to the female patients.

Rheumatoid factor was present in 20/55 S.L.E. cases tested, and non-organ-specific A.I.C.F. reactions were obtained in 26 of the 65 cases.

Rheumatoid Arthritis

Of the 86 rheumatoid arthritis patients, 16 had positive L.E. cell tests and A.N.F. was found in 43. Results of tests for thyroid antibodies in this group are shown in Table II. As in the former group, high titres were found only in the seven patients with associated myxoedema or Hashimoto's disease. Of the remaining 79 cases, nine gave positive tests, all of low titre. Three of these belonged to a group of seven cases associated with colloid goitre or thyrotoxicosis. The other six were in the group of 72 rheumatoid arthritis patients without thyroid involvement. This incidence corresponds closely with that found in non-rheumatoid groups—that is, the presence of colloid goitre increases the likelihood of thyroid antibodies from the normal of about 10% in females of that age group to 30–40%. Latex sheep-cell tests were positive in 39 of 70 cases tested, and A.I.C.F. titres of 1/16 or over were obtained in 8 of 78 cases tested, one occurring in a patient with combined R.A. and Hashimoto's thyroiditis.

TABLE II.—*Incidence of Thyroid Antibodies in R.A. Patients With and Without Thyroid Involvement*

	Thyroid Antibodies		Totals
	+	−	
Without colloid goitre or thyrotoxicosis	6	66	72
With colloid goitre or thyrotoxicosis ..	3	4	7
,, Hashimoto's thyroiditis	7	0	7

Hashimoto's Disease

The 182 uncomplicated Hashimoto cases had negative L.E. cell tests, mostly done by the indirect method. Nuclear complement-fixation tests were also negative in 100 cases tested. Weak antinuclear reactions were obtained by the Coons technique in 14 of 182 cases when the staining procedure was carried out at room temperature. These sera were positive in low titre, in the range 1/10 to 1/20, and when the tests were repeated at 37° C. reactions became very weak or negative. (Couchman *et al.*, 1961, have noted this effect with some A.N.F. positive sera from miscellaneous hospital cases and pregnant women.) No effect of temperature on A.N.F. positivity was found with S.L.E. or R.A. sera.

The latex test for R.A. factor was positive in two of 98 uncomplicated Hashimoto cases tested and was positive in four of the seven patients with combined R.A. and myxoedema or Hashimoto's disease. A.I.C.F. reactions against human liver were found in 7% of the 182 thyroiditis cases tested, and titres ranged from 1/4 to 1/32 except for one patient who gave partial fixation up to 1/128. When only titres of 1/16 or greater are considered, the incidence of A.I.C.F. in Hashimoto's disease is 5%, and the reaction was combined with a positive A.N.F. test in only one instance.

Control Groups

A recent hospital population study (Hill, 1961) using our T.R.C. and C.F.T. methods showed an incidence of 10% thyroglobulin antibodies in women aged between 40 and 60. Using 2 M.H.D. of complement and 1/4 dilution of serum, we have found no thyroid-specific complement-fixing antibodies in non-thyroid patients, but if the Coons technique with neat serum is employed cytoplasmic staining is found in 8% of middle-aged women. A.I.C.F. reactions were found in less than 1% of a mixed hospital population, but exact figures are difficult to give owing to variability of the antigens. The A.N.F. test gave weak positive results in 2 out of 132 normal sera and in 2 out of 65 cases of pulmonary tuberculosis tested at Taplow using infant thyroid, and in 18 out of 560 (3.2%) mixed thyroid patients excluding Hashimoto and myxoedema at the Middlesex Hospital, using fresh thyrotoxic thyroid as substrate. The latex test in our hands gives positive results in <2% of patients showing no joint disease. The L.E. cell test performed in Leyden gave negative results in over 3,000 normal subjects and mixed hospital patients.

TABLE III.—*Serological Findings in Seven Cases of Combined Liver and Thyroid Disease*

Case	Sex and Age	Diagnosis	Nuclear Antibodies		Thyroid Antibodies			A.I.C.F.	Rh Factor
			L.E. Cells	A.N.F.	T.R.C.	Pptin.	C.F. Titre with Thyroid Microsomes	C.F. Titre with Liver Microsomes	Latex
1	F 66	Cirrhosis+Hash. dis.	−	±	2,500	+	128	−	−
2	F 40	Lupoid hepatitis+myxoedema	+	±	2½ m.	+	32	−	−
3	F 43	Cirrhosis+Hash. dis.	−	−	2½ ,,	+	>512	64	−
4	F 28	Lupoid hepat.+myx.	+	−	2½ ,,	−	32	32	−
5	M 70	Prog. hepat.+Hash. dis. ..	n.d.	−	2½ ,,	+	128	64	+
6	F 43	Lupoid hepat. pulm. fibrosis, +Hash. dis.	+	−	− (CA2+)	−	128	32	−
7	F 50	Cirrhosis+Hash. dis.	n.d.	n.d.	25,000	+	−	4	n.d.

n.d.=Not done.

TABLE IV.—*Comparison of Complement Fixation Reactions with Subcellular Fractions of Human Liver and Thyrotoxic Thyroid*

Case	Clinical Diagnosis	Thyroid		Liver				Remarks
		Whole Homogenate	Micro-somes	Nuclei	Mito-chondria	Micro-somes	Super-natant	
1	S.L.E.	32	0	32	512	128	512	
2	,, ...	⩾512	512	⩾512	⩾512	⩾512	128	T.R.C. titre 2,500. Patient not avail. for clin. invest
3	,, ...	128	—	—	—	—	128	Serum anti-complementary to 1:32
4	,, ...	128	0	16	128	0	32	
5	,, ...	256	0	0	128	32	16	
6	,, ...	512	0	0	256	16	512	
7	R.A.	256	0	0	256	256	256	L.E. cells
8	Lupoid hepatitis+myxoed.	32	32	8	256	32	0	L.E. cells Thyr. pptin.
9	Cirrhosis	512	0	0	>512	>512	>512	
10	? Mild L.E.	64	0	8	>512	256	8	A.N.F.+L.E. cells. Low serum complement
11	Post-op. colloid goitre ..	>512	0	0	>512	>512	>512	A.N.F. negative. No symptoms
12	Hash. dis.	>512	32	8	32	8	>512	T.R.C. titre 25,000
13	Toxic Hash. goitre: malig. exophth.	>512	>512	0	8	8	0	Thyr. pptins.
14	Hash. dis.	128	16	64	>512	256	16	,, ,,

Liver Disease With Overt Thyroiditis

The seven patients in this group all had progressive hepatitis or cirrhosis of unknown origin with liver failure. Five also had Hashimoto goitres and two had myxoedema without thyroid enlargement. Details of serological findings are shown in Table III. Thyroid-specific antibodies were of high titre, and five out of seven cases also gave C.F.T. with human liver. Three of the cases had given occasional positive L.E. cell preparations, but were subsequently treated with cortisone, and we were not able to obtain convincing A.N.F. results on the specimens taken after prolonged treatment. A more detailed analysis of sera giving C.F. reactions with thyroid and other organ extracts is presented in Table IV. The majority of the S.L.E. sera were negative when tested with thyroid microsomal fractions which contained the organ-specific cellular antigens; where positive results were obtained there was clinical or serological evidence of thyroiditis. These sera were tested together against subcellular fractions prepared from the same liver. The results show that several antigens must be implicated in the A.I.C.F. reaction and that the antibody spectrum varies widely from one patient to another (cf. Deicher, Holman, and Kunkel, 1960).

Discussion

This study of systemic lupus erythematosus, rheumatoid arthritis, and thyroid auto-immune disease indicates that between these diseases there exists a degree of overlap of auto-antibodies greater than might be expected to occur by chance from control studies. Thus in our patients with S.L.E. the incidence of thyroid antibodies was approximately three times higher than in control subjects of the same age and sex. The antibodies were of low titre, and were detectable only by the sensitive tanned-cell test or, in the case of the second colloid antigen, CA2, by the fluorescent test. Although in this group C.F.T. results with homogenates of thyrotoxic glands were positive in 40% in high titre, reactions were almost invariably attributable to non-organ-specific antibodies, which reacted equally well with liver extracts but gave negative results with thyroid microsomal preparations. The five cases with combined disease (S.L.E. with Hashimoto's disease) gave the results expected—that is, antibodies specific for thyroid antigens, and antibodies typical of systemic lupus. In the rheumatoid arthritis group without goitre the incidence of thyroid antibodies was comparable to that found in controls. Three of the seven patients with goitre had low-titre antibodies corresponding with the incidence found in similar thyroid disease without R.A. Of the 79 R.A. patients without overt thyroiditis only two reacted in the C.F. test with thyroid microsomes, while seven sera gave A.I.C.F. reactions with human liver to titres of 1/16 or over. The incidence of this non-organ-specific reaction is thus much lower in R.A. patients than in the S.L.E. group. Buchanan *et al.* (1961) found thyroid complement-fixing antibodies in 12 of 46 female rheumatoid patients, whereas only three of their 73 cases had positive A.I.C.F. reactions. In female control cases these workers found thyroid C.F.T. in 11% as compared with the almost negative results of our tests for this group. This discrepancy may be partly explained by the greater sensitivity of the C.F. test used by Buchanan and co-workers in relation to serum concentration and amounts of complement. Yet, despite the greater sensitivity of their C.F. test, these workers find a lower incidence of A.I.C.F. in rheumatoid patients than was obtained in the present study. The possibility should therefore be considered that extracts of post-mortem adrenal, kidney, and liver used as antigen are not as sensitive as fresh thyroid gland for detecting the non-organ-specific A.I.C.F. reaction. In our opinion it is desirable to confirm the thyroid specificity of sera by testing them with thyroid microsomal antigen and also by demonstrating cytoplasmic fluorescence of thyroid cells with the Coons technique.

Our seven cases of combined R.A. and Hashimoto's disease were drawn from a series of over 300 Hashimoto sera tested, excluding cases of Sjögren's syndrome. This incidence is much lower than that reported by Beare (1958), who found three cases of R.A. among 27 cases of proved Hashimoto goitre in the London area. Buchanan *et al.* (1961) also found a higher incidence—five out of 31. We cannot at present explain the differences in incidence of the combined diseases found by us. It is possible that we were not always informed of other diseases present in patients sent to us for thyroid antibody tests. This seems unlikely, since the latex test for rheumatoid factor was performed on 98 of the Hashimoto sera and was found positive in only two.

It is noteworthy that in both the S.L.E. and uncomplicated R.A. groups the incidence of thyroid antibodies was confined to female patients, and that the occurrence of Hashimoto's disease in association with either S.L.E. or R.A. was found only in women. In contrast, the men in these two groups showed the same incidence as the women of A.I.C.F. reactions, A.N.F. and R.A. factor, and this suggests that thyroid antibodies might stem primarily from some abnormality in the thyroid itself rather than from an abnormal immunological reactivity in general.

In the great majority of *Hashimoto cases,* auto-immune phenomena were directed exclusively against the thyroid gland, and in the uncomplicated thyroiditis cases no L.E. cells were found. In a small proportion the spectrum of antibodies was wider, but reactions were of low titre, their incidence was little or no higher than in comparable control groups, and they were without apparent clinical significance.

The cases of thyroiditis combined with progressive hepatitis (Table III) showed coexistence of complement-fixing antibodies to both non-organ-specific antigens and the specific thyroid microsomal fraction.

The results reported here are in line with previous serological studies in human disease and in animal experiments, which suggest that auto-immune diseases involving body constituents might be broadly classified into two main categories (Mackay and Larkin, 1958; Asherson and Broberger, 1961). These could be designated "disturbed antigen" and "disturbed tolerance" diseases, exemplified on the one hand by Hashimoto's thyroiditis and on the other by systemic lupus erythematosus. In "disturbed antigen" diseases the relevant organ constituents are considered to be inaccessible to the lympho-reticular system and so fail to establish immunological tolerance in early life. Exposure of these substances to lymphoid elements after immunological maturity as a result of leakage from the tissue could evoke an immune response without implying marked abnormality of the R.E. system. This view is supported by the fact that these organ-specific antigens are able to evoke auto-antibody production in normal animals, as has been demonstrated with brain, testis, thyroid, adrenal, uveal tract, and lens constituents.

Furthermore, immunization with these materials often produces organ lesions in the injected animals resembling the parallel human disease (Waksman, 1959).

In contrast, the antigens characteristically involved in lupus erythematosus are widely distributed in body components, and, in the case of D.N.A.-protein for example, are even present in the antibody-forming cells themselves. Tolerance to them must therefore be presumed to exist in the normal state. These antigens fail to provoke auto-antibody production in experimental animals under conditions known to succeed with the organ-specific constituents, and lupus lesions have never been actively induced in animals. Burnet (1959, 1961) distinguishes between auto-immune diseases derived from defects of the antibody-forming cells and those depending upon some alteration in the distribution of normally inaccessible antigens. On the basis of his clonal selection theory he visualizes the emergence of forbidden clones arising by mutation and surviving by failure of an unknown homoeostatic mechanism to eliminate them. In the case of inaccessible antigens, the relevant clones are not eliminated in early life by the required contact with antigen.

Support for the concept that the underlying disturbances involve the antigen in one group of diseases and the antibody-forming system in the other is provided by family studies in the relatives of Hashimoto patients. Thus the high incidence of various thyroid disorders, such as colloid goitre and thyrotoxicosis as well as lymphadenoid goitre and primary myxoedema, suggests a constitutional abnormality of the thyroid gland, which seems to find expression in the greatly increased frequency of thyroid antibodies in these families (Hall *et al.*, 1960; Doniach *et al.*, 1961). In contrast, in the relatives of lupus patients there is a familial tendency to aberrations of γ-globulin synthesis such as hypergammaglobulinaemia (Leonhardt, 1959), and agammaglobulinaemia (Kunkel, 1959).

The differences and similarities between the two suggested categories of auto-immune diseases are summarized in Table V. While this division may provide a useful working hypothesis to approach the complex mechanism underlying the development of auto-immunity, it clearly represents an oversimplification. The two diseases lymphadenoid goitre and systemic lupus appear to be extreme examples at opposite ends of a spectrum of diseases showing varying immunological disturbances. Thus most other human diseases which have been linked with auto-immune phenomena, such as colitis (Broberger and Perlmann, 1959), pancreatitis (Thal, Murray, and Egner, 1959), progressive hepatitis (Mackay and Larkin, 1958), haemolytic anaemias (see Dacie, 1954), Sjögren's syndrome (Jones, 1958; Heaton, 1959; Bloch *et al.*, 1960; Bunim, 1961) and rheumatoid arthritis (see Glynn and Holborow, 1960), show a variable incidence of complex auto-antibody patterns, some of which are commonly found in S.L.E.

TABLE V.—*Comparison of Two Categories of Auto-immune Disease*

"Disturbed Antigen" Disease	"Disturbed Tolerance" Disease
Exemplified by Lymphadenoid Goitre	Exemplified by S.L.E.
Differences	
Antigen not normally circulating	Antigen accessible to lymphoid cells
Immune tolerance to antigens probably not established	Tolerance to antigens established in early life
Organ-specific antibodies produced in patient	Antibodies not necessarily organ-specific
Antibodies have narrow species-specificity	Antibodies have wide species-specificity
Narrow spectrum of antibodies produced	Often a wide variety of antibodies in patient's serum
Antigens capable of evoking antibody response in healthy animals	No antibodies produced in animals with comparable stimulation
Experimental lesions resemble human disease	Human disease difficult to reproduce in animals
Relatives of patient have tendency to several thyroid diseases	Relatives have disturbances of γ-globulin synthesis
Similarities	
Circulating auto-antibodies to normal body constituents	
Tendency to raised level of serum γ-globulin	
Lymphoid invasion of affected organs	
Variable damage to cells containing auto-antigens.	
Greater incidence in women.	
Disease process not always progressive (cf. discoid L.E. and focal thyroiditis)	
Circulating antibodies not primarily responsible for tissue damage (except presumably in haemolytic anaemia), but may act synergistically with "delayed type" hypersensitivity reactions	

These complex patterns include three classes of antibodies: those involving organ-specific antigens, A.I.C.F. reactions involving non-organ-specific cytoplasmic antigens, and the group of antinuclear factors. Antinuclear factors may reasonably be taken as a criterion of disturbed immunological tolerance. They are found in practically all cases of systemic lupus, in a high proportion of Sjögren cases, and to a lesser extent in other connective-tissue diseases. They have also been reported in progressive hepatitis, in hydrallazine syndrome, and in drug hypersensitivity; weak reactions occur occasionally in Hashimoto's disease. Nuclear antibodies have not yet been looked for in chronic pancreatitis, and we have tested for A.N.F. only five cases of adult ulcerative colitis, with negative results.

The A.I.C.F. reaction is not fully analysed with regard to the antigens involved. The group of antibodies reacting with various organ extracts is certainly very complex (Asherson, 1959), and some of the antigens may be organ-specific. It is not yet clear whether the A.I.C.F. reaction results from a defect in the antibody-forming system or whether "secluded" antigens common to a number of organs are released by cell injury. There is evidence for the latter possibility in the transient liver haemagglutinating or C.F. antibodies found in many patients after infective hepatitis (Haven, 1958), in cardiac infarction (Ehrenfeld *et al.*, 1961), and in rats following injections of carbon tetrachloride (Weir, 1961). It would be relevant to see if sera giving high A.I.C.F. titres in the absence of A.N.F. would react with leucocyte extracts, since the constituents of these cells presumably induce full tolerance in early life. In diseases of auto-immune pathogenesis where lesions are localized to one organ the cell destruction may be mediated primarily by the "delayed hypersensitivity" component of an immune reaction involving organ-specific antigens, and the non-specific A.I.C.F. antibodies may be a phenomenon secondary to this injury.

Sjögren's syndrome provides evidence for a combination of disturbed tolerance, as shown by the high incidence of antinuclear factors and of rheumatoid phenomena, together with an organ-specific destructive lesion in the salivary and lacrimal glands, though no specific antigens have yet been isolated from these organs. Curiously, these patients have a higher incidence and higher titres of thyroid-specific antibodies than are found in S.L.E. (Bloch *et al.*, 1960).

A disturbance of immunological tolerance could theoretically increase the likelihood of an immune response to organ-specific antigens, either by reducing the threshold of response to sporadic minute leakages from the relevant organ, or possibly by increasing such leakage through tissue damage resulting from inflam-

matory response to deposits of nuclear antigen-antibody complexes. The increased incidence of thyroid antibodies in the S.L.E. patients could arise in this way. The leakage theory may account for the antibody response to "secluded" antigens, but further as yet unknown factors must be sought to explain the sustained and progressive organ destruction so often observed in human auto-immune diseases. Progression of the lesions is not usually seen in the experimental auto-allergies induced in animals with organ-specific antigens in Freund adjuvants.

The mechanisms of immunological tolerance which prevent antibody formation against recognized self-constituents are still unknown, and several hypotheses have been discussed recently (for example, Asherson and Broberger, 1961). Where tolerance is already established it could break down either after some abnormality in the cells of the antibody-forming system, or because of modification of the antigens (? caused by mutations or virus and bacterial components). A small alteration in the metabolic degradation of a body constituent in the reticulo-endothelial system could expose new antigenic determinants. This may be one of the mechanisms by which Freund adjuvants enhance auto-antibody production in animals.

Our present ignorance of the basic mechanisms of immune tolerance and its breakdown in disease precludes the formulation of a well-defined theory of auto-immune diseases, but it is felt that the study of clinical and serological relationships in this group of disorders will increase our understanding of their development and progression and may ultimately shed some light on the disturbances which initiate them.

Summary

Patients with systemic lupus erythematosus, rheumatoid arthritis, and Hashimoto's disease, and with combinations of these conditions, were investigated for auto-antibody production. Tests were carried out for thyroid-specific antibodies, for the non-organ-specific antibodies giving the auto-immune complement-fixation (A.I.C.F.) test of Gajdusek, for L.E.-cell formation and antinuclear factors, and for rheumatoid factors (latex test). Thyroid antibodies were present in high titre in all the sera from the cases of Hashimoto's disease; in females with S.L.E. they were found, although in low titre only, with about three times the frequency observed in controls of comparable age and sex.

The incidence of thyroid antibodies in rheumatoid arthritis was confined to women, was not significantly higher than in the control group, and was similarly influenced by the presence of goitre.

The A.I.C.F. test was positive in 37% of the S.L.E. cases, in 10% of cases of rheumatoid arthritis, and in 5% of Hashimoto's disease. L.E. cell and A.N.F. tests were positive in all but one of the S.L.E. cases, and these two tests were positive, respectively, in 20% and 50% of the rheumatoid cases.

In Hashimoto's disease L.E. cell-tests were consistently negative and A.N.F. was seldom present, and then only in low titre. Latex tests were positive in 40% of S.L.E., and 55% of R.A. cases, and negative in Hashimoto's disease. The combination of Hashimoto's disease with S.L.E. or with rheumatoid arthritis was encountered only in women, and positive auto-antibody tests in these cases were distributed with frequencies approximately related to those found in each condition separately.

These findings are discussed. They appear to fit the concept that auto-immune diseases form, with some overlapping, a broad spectrum, at one end of which the immunological disturbance concerns mainly antigen, and at the other involves mainly antibody production.

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